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Agricultural Research

CURRENT SERI
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FIRE ANTS
USDA Team Lends
a Hand in Brazil



Solving Problems, Meeting Needs

The Agricultural Research Service is often called on to help solve urgent problems beyond the regular routine of its everyday research.

A recent example is the team of ARS scientists helping combat a fire ant population explosion in a remote Brazilian town (page 4). In this case, the short-term lending of ARS scientific expertise, coupled with corporate donation of an appropriate technology, may be all that is needed to bring relief to a beleaguered community.

This is technology transfer on a human scale. ARS response to such external requests for aid has been as generous and consistent through the years as resources have allowed. This is part of what the "service" in our agency's name implies—seeing to it that practical applications of our research emerge from the laboratory and find their way to a wide range of users.

Of course, responding to one particular group of extra-agency research needs is more than just good-heartedness—it's a mandate: One of the major parts of the ARS mission is to carry out research that supports action and regulatory agencies charged with enforcing federal regulations and laws.

Currently, these include nine USDA agencies: the Agricultural Marketing Service, Agricultural Stabilization and Conservation Service, Animal and Plant Health Inspection Service, Federal Grain Inspection Service, Food and Nutrition Service, Food Safety and Inspection Service, Forest Service, Human Nutrition Information Service, and the Soil Conservation Service.

Three non-USDA agencies and departments we serve are the Environmental Protection Agency, Food and Drug Administration, and Department of Defense.

ARS can't solve every problem it's asked to solve, so agency program planners review them and choose which ones to tackle based on whether the problem is researchable, solutions might already exist from previous research, ARS is capable of performing the requested research, resources to support the work are available, and solving the problem is compatible with the ARS mission. That we are able to shift expertise and resources to address these special problems is due to our broad-based fundamental research program and flexible planning.

Current ARS research programs dealing with needs of action and regulatory agencies include:

Food safety and nutrition—

- identify multiple residues in food animal tissues;
- reduce foodborne bacteria, viruses;
- show the temperature to which a product has been cooked or whether it has been irradiated;
- improve control of contaminating insects and parasites;
- refine methods to assess the nutritional status of individuals;
- determine and enhance nutrient bioavailability;
- identify nutritional components that reduce the risk of cancer, diabetes, obesity, and other chronic disorders.

Pesticides and the environment—

- control aquatic and other serious weeds with integrated pest management;
- minimize applications that harm beneficial species;

- perfect biocontrol methods and genetically resistant strains to reduce losses from agriculturally important pests and diseases.

Range and forage management—

- develop low-input forage production techniques and varieties;
- improve rangeland management methods to enhance the quantity and quality of water supplies;
- devise economically sound livestock foraging systems to help keep highly erodible lands under protective cover;
- evaluate effects of grazing systems on soil, hydrology, and productivity.

Animal health—

- develop vaccines for avian influenza and brucellosis;
- improve diagnosis of avian influenza, bluetongue and other orbiviruses, bovine lentivirus, scrapie in sheep and goats, and other diseases of economic importance to producers.

Commodity quality enhancement—

- detect and quantify mycotoxins and chemical residues in stored grain;
- refine rapid, objective classification methods for wheat;
- create a biochemical test for insect parts in whole grains;
- devise a simple way to quantify starch and fiber in feed grains;
- design an automated grain inspection system;
- fine-tune instruments to measure cotton fiber quality quickly and accurately, grade for objective evaluation of meat animal carcasses, and nondestructively assess the ripeness of fresh fruit .

Jesse W. Goble
ARS Budget and Program
Management Staff

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Agricultural Research



Cover: Imported red fire ants scurry across a Popsicle stick after being disturbed by scientists at ARS' Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida. Widely disliked for their venomous, painful stings, fire ants have spread across much of the southern United States. Photo by Scott Bauer. (K-5388-1)



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Fighting the Fire Ant

Brazilian trip shows persistence of pest now found across the U.S. South.

Sounds like something out of an old-time science fiction movie: Millions of aggressive, stinging ants overrun a small city in the Amazon rain forest, invading homes, schools, churches, and stores. Nothing seems to stop them.

But to the people of Envira, Brazil, it's not a movie script. It's real life.

There, in the Amazon rain forest, the 10,000 people who call this small, riverbank town home have been struggling against a booming population of fire ants. It started several years ago, when people noticed more and more of the tiny, reddish-brown, omnivorous insects foraging for food. Unable to stop them, the Brazilian government asked for help from fire ant experts in the U.S. Department of Agriculture.

In September 1993, three USDA researchers—entomologist David Williams and agricultural engineer Danel Haile of the Agricultural Research Service, and entomologist Homer Collins of the Animal and Plant Health Inspection Service (APHIS)—went to Envira, armed with 2,000 pounds of bait containing an insect growth regulator that inhibits the development of worker fire ants. The bait, called Logic, was donated by the chemical manufacturer Ciba-Geigy.

The scientists went not only to help the authorities control the pest, but also to try to determine why fire ants—which now also infest the southern United States—had taken hold to such an extent in Envira, an otherwise typical town in the tropics.

"It's not an exaggeration to say that the ants were overrunning the place," says Williams, echoing a sentiment shared by Haile and Collins. "We found some colonies with more than a million ants inside. I've been studying fire ants for 17 years, and I've never seen anything like it."

The town's plight has drawn international media attention, as well

HOMER COLLINS/APHIS



Unnatural imbalance: Cleared land in the Amazon rain forest outside Envira, Brazil, created an ideal environment for fire ants, which thrive when habitats are disturbed. Scientists say development may be one factor in fire ant population explosions.

as blanket coverage inside Brazil. According to one report in the Washington Post, the ants have attacked vegetables, animals, and people and have killed chickens, ducks, and turtles. Several children were hospitalized after suffering severe reactions to fire ant stings. Like bee venom, the ant venom contains proteins that can cause anaphylactic shock and, in extreme cases, even death.

"Almost every family has had children attacked," the town's Mayor, Luiz Castro, was quoted as saying. If the problem cannot be solved, "Many people are saying they will sell their things and go.... They are discouraged. Your children can't go out and play. You can't grow chickens. My son even had bites in his ears."

The three scientists have seen serious fire ant infestations before, in the United States. Williams, the team



leader, is an entomologist at the ARS Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida. He's studied the ant extensively in the United States and in its native South America. Haile, an agricultural engineer, is also based at the Gainesville lab and provided expertise in bait application technologies. Collins, an entomologist, is located at the APHIS Imported Fire Ant Station in Gulfport, Mississippi, and has worked on fire ant

control and quarantine projects in the United States. The Gainesville and Gulfport sites are USDA's only fire ant research stations.

Despite their experience with the pest, they weren't prepared for what they saw in Envira when they landed at the small airstrip on September 10. "No sooner had we stepped off the plane than we were surrounded by about 8,000 people—almost the town's entire population," Williams says. "We

realized then the extent of the problem—how seriously they viewed it."

The next day, the scientists began looking for fire ant mounds, and it didn't take long before they found some. There were thousands—and in a dry river bed that runs through the town, they found the largest ones.

"We found some mounds there that were about the size of my desk," Williams says. "It was a sight to see. There were easily more than a million ants in each one. They were the biggest fire ant colonies I've ever seen."

On September 13, at a town meeting, dozens of people came forward to ask questions. "We reassured them that the insect growth regulator is safe to people and livestock. We also explained how it works and how long it would take to control the ants," Williams says.

They began spreading Logic, a granular material, on as much of the 2,400-acre town as they could with a tractor—and hand spreaders in areas where the tractor could not be used. "The ideal way would have been by airplane, but the area was so remote that it wouldn't have been practical," Haile says. "And it would have been too expensive that way."

They spread about 500 pounds of Logic and treated individual fire ant mounds. Logic contains the active ingredient fenoxy carb, which inhibits development of worker fire ants. The chemical is dissolved in soybean oil that is then applied to corn granules.

Fire ant workers carry the granules—about the size of a grain of coarse sand—back to their nest and feed them to the queen. She continues to lay eggs, but they never develop into worker ants. Eventually, workers that are present die and are not replaced. Without workers, the queen also dies and the colony collapses.

Williams says the remaining 1,500 pounds of chemical will be used for additional treatments during the rainy

season in February 1994 and again later as needed. The USDA team has trained Brazilian scientists to make those applications, and the Brazilian researchers will teach Envira citizens to treat individual ant mounds. Without these efforts, the ants will return.

Where'd They All Come From?

How were the ants able to take over Envira? The researchers say there are several key reasons.

One was the rapid growth in the area over the last decade. Between 1980 and 1993, the town's population mushroomed from an estimated 1,000 to 10,000. As the city expanded, more land was cleared for new housing, streets, and other construction. As the native forest and habitat were destroyed, the fire ant population grew.

"Fire ants thrive whenever the native habitat is disturbed," Williams says. "That's because they reproduce

so quickly and can out-compete other ants that are less aggressive stingers."

They also have the ability, unique among ants, to float on water.

Envira is located along two tributaries of the Amazon, and during the wet season, the rivers flood. Rising water drowns many ants, but fire ant workers form themselves into a ball—the size of a softball or even a soccer ball—around the queen to protect her. As the water rises, they float on top of it like a boat, Williams says.

"When they bump into something, they climb onto it," he says. "If that something happens to be a house, they'll nest in the house. For fire ants, floods aren't a danger but are simply a way for them to move around."

The researchers also say that, based on what they were told by local authorities, fire ant populations began to boom several years ago when the government stopped spraying DDT to control malaria-causing mosquitoes. Also, until recently, trash wasn't properly disposed of—providing a ready food supply for the ants, which will eat just about anything.

"With all those factors at work, it's not hard to see why the ant population took off," Williams says.

But the researchers could not conclusively determine a key biological factor: whether the mounds in Envira contained multiple queen colonies. Fire ant nests usually contain only one queen, which can produce her weight in eggs—about 2,000—every 24 hours, Williams says. But, over the last decade, researchers have discovered colonies where workers will accept more than one queen—meaning much higher ant populations.

"We're pretty sure that the larger colonies, with more than 500,000 ants, had multiple queens," he says. "I dug into one and found a queen right away. Normally you'd have a hard time finding the queen if there was only one,

SCOTT BAUER



Small but feisty: Similar to the fire ants in Brazil, this laboratory colony of red imported fire ants gets a close inspection from entomologist David Williams (left) and agricultural engineer Dan Haile. (K5386-15)



To the rescue: USDA researchers received a lot of attention when they landed in a Brazilian Air Force cargo plane at the Envira airstrip.

because they're pretty well protected deep inside."

Williams said they were unable to confirm multiple-queen colonies

DAN HAILE/ARS



Ouch! Multiple fire ant stings caused the sores on this young resident's leg and foot.



primarily because it was so difficult to study them. "It was hard to do much poking around in the ant mounds," he acknowledges. "I was stung 50, maybe 75 times in the course of probing about five or six ant mounds. I was jumping around quite a bit."

"When I would poke a mound with a shovel, ants would come up from inside tiny tunnels that can extend 50, perhaps 100 feet out from larger colonies," he adds. "They're more aggressive than the fire ants we know in the United States. Within a few seconds of poking a nest, the ants come right out of the tunnels under your feet and start attacking. You have no time to react."

Based on preliminary studies, the researchers believe the fire ant species in Envira is *Solenopsis saevissima*, commonly found in South America, and a sister species of *S. invicta* that infests much of the southern United States, from Texas to Florida and as far north as Tennessee and Norfolk, Virginia.

Cooperating scientists are conducting further genetic tests to confirm that the species from Envira is indeed *S. saevissima*.

What the scientists don't know is why Envira had such an overwhelming fire ant population, while other cities in the Amazon basin apparently don't

HOMER COLLINS/APHIS



Home base: ARS scientists David Williams (left) and Dan Haile found a huge mound of earth—about 10 feet long—occupied by a giant colony of fire ants in a dry river bed leading into the Tarauaca River in background. During the wet season, floods drive some ants upward and closer to people's homes, while others float downstream in buoyant, living balls.

have similar trouble. "Other towns may have a problem but haven't reported it, since many of them are so remote," Williams says.

The researchers have not yet made plans to return to Envira, but would like to go back to learn more about the fire ant infestation there and to see if the bait applications have worked. Once the initial supply of bait is used up, Envira officials say, they don't have money to buy more.

"One thing is clear: If the Brazilians don't continue to treat, the fire ants will be back," Williams says.

Fighting the Fire Ant at Home

There are no Envira's in the United States when it comes to fire ants. But the pests have caused their share of trouble here since they were accidentally imported from South America more than 50 years ago.

They now infest 11 southern states and Puerto Rico, and they have been found in, but eradicated from, several western states.

Like other foreign invaders that enter the United States, imported fire ants have thrived here in the absence of natural enemies and as development has disturbed the habitats of competitive native ants.

Eradicating them from the United States is unlikely because they are so widespread, but they can be managed so they aren't as much of a problem, U.S. Department of Agriculture scientists say.

Richard Patterson heads the fire ant research unit at Gainesville.

He says scientists there are studying the basic biology and behavior of the ants and are working on chemical repellants, baits, and biological control organisms.

Biocontrol involves finding natural enemies in the ants' native South America and bringing them back to this country. One of the most promising candidates is *Theholania solenopsis*.

It is a type of protozoan, called a microsporidian, that researchers discovered in South America in the 1960's. Its spores infect fire ant fat and blood cells, eventually weakening the ants and killing the colonies.



Fire ants feed on laboratory ration. (K5387-14)

Patterson and ARS entomologist Juan Briano at the agency's South American Biological Laboratory in Hurlingham, Argentina, conducted a 4-year field study there from 1989 to 1993 using *T. solenopsis* against the fire ant species *Solenopsis richteri*.

The result: 85 to 90 percent of the colonies died after being infected with the biocontrol protozoan. And surviving colonies were weakened and reduced to sizes 70 to 80 percent smaller than normal, Patterson says.

T. solenopsis' biocontrol potential was boosted late in 1993 when Briano, in lab tests in Argentina, was able to infect *S. richteri* fire ants by feeding them the organism directly. "Previously, we thought the ants could only be infected indirectly through what we call an intermediate host," Patterson says. "If that had been the case, it would have made it much more complicated to use the organism as a biocontrol agent."

Patterson says the protozoan can be transmitted either through food or from the queen to her eggs—making its potential even greater. "If we can infect the queen, then all of her offspring will be infected," he says. "That makes control even more effective."

Further studies are being undertaken to determine that *T. solenopsis* will kill the fire ant species *S. invicta*, the problem ant in the United States. *S. invicta* and *S. richteri* are related species, and Patterson expects the protozoan to be effective against both. Scientists are planning additional lab studies in 1994 and field studies in 1995, before seeking regulatory approval to use *T. solenopsis* in the United States.

In other fire ant research under way at Gainesville:

- Williams has evaluated 33 chemicals as fire ant repellants and found several that look promising. Scientists can't give specifics yet, because ARS plans to seek patents on

HOMER COLLINS/APHIS



Where tractors couldn't go: Workers applied Logic, an insect growth regulator, with hand-held sprayers in the dry river bed.

them. Several major chemical companies are also interested in setting up formal cooperative research and development agreements to commercialize these repellants.

In other work on repellants, "a patent application has been submitted covering several chemical classes of repellants," says Robert K. Vander Meer, a chemist at the Gainesville lab. "We also have formal research and development agreements with industry aimed at devising slow-release formulations of the repellants. So far, we've been able to extend the effective repellent life to 6 months."

Currently available fire ant bait toxicants harm nontarget ant species, making it easier for fire ants to reinfest an area. "We are developing phero-



mone-enhanced baits to better direct the bait toxicant to the fire ant," says Vander Meer. This leaves the "good" ants to act as predators of newly mated fire ant queens and to compete with encroaching fire ant colonies.

"We have a patent application in preparation and some of the work is supported through a cooperative agreement with industry," Vander Meer says.

• A 21-year study of nearly 1 million ants in the Gainesville area confirms that imported fire ants are continuing to spread, displacing native ant species and causing a large number of other ecological problems. *S. invicta* was found in 65 of 100 traps in 1992, compared to only 1 trap when the study

began in 1972, says entomologist Daniel P. Wojcik.

The study was the first to gauge the impact of the pest in an area that has not received large-scale insecticide treatments. In the study, scientists collected 990,079 ant specimens—representing 55 ant species—in 100 roadside traps containing meat or honey-based baits.

"The survey confirms that fire ants are having a tremendous ecological impact in this area," Wojcik says. "Pest control operators now identify fire ants as the number-one indoor ant pest problem in north Florida."

Wojcik says the pest is having a similar impact elsewhere in the southeastern United States. "We've conducted unpublished studies showing that

the pest is causing problems in Texas, Georgia, Alabama, and Mississippi."

• Scientists have discovered that a fire ant foraging for food finds its way back home by using a sort of internal compass that senses the earth's magnetic field. "This is the first time this phenomenon has been found in ants," says Vander Meer.

A compass-like material, called magnetite, apparently allows the fire ants to sense the normal south-to-north direction of the earth's magnetic field. "When we altered the normal magnetic field in total darkness, it took the ants more than twice as long to form a trail to a food source," he says. He and former University of Florida scientist James B. Anderson made the discovery during a recent 4-year study.

Vander Meer says the fire ant may also use its magnetic sense for orientation inside the dark confines of its nest. The fire ant has already been reported to be attracted to electrical fields found in air conditioning units, telephone switch boxes, traffic light signals, and other areas where electricity is used.

"We are investigating the possible relationship between the ant's magnetic sense and its attraction to electrical current, because for every current there is an associated magnetic field," Vander Meer says. "It may be possible to attract fire ants to a toxic bait using a magnetic or electrical field."—By Sean Adams, ARS.

Richard S. Patterson, David Williams, Daniel P. Wojcik, Daniel Haile, and Robert K. Vander Meer are at the USDA-ARS Medical and Veterinary Entomology Research Laboratory, P.O. Box 14565, Gainesville, FL 32604; phone (904) 374-5910/5982/5986/5928/5918, fax (904) 374-5818.

Homer Collins is at the USDA-APHIS Imported Fire Ant Station, Gulfport, MS; phone (601) 864-0120, fax (601) 864-9956. ♦

Oat Chromosomes on Display

Computers and video cameras could help oat breeders trim years off the time needed to develop an oat plant resistant to crown rust, a disease that in 1991 cost growers in Iowa, Minnesota, South Dakota, and Wisconsin an estimated \$17 million.

ARS plant geneticist Howard W. Rines and University of Minnesota researchers in St. Paul are using these electronic tools to get a clearer picture of what oat chromosomes look like.

"Through a microscope, using conventional staining techniques, oat chromosomes look pretty much alike," Rines says. "Until now, we could only tell the long ones from the short ones."

Four years ago, Rines and University of Minnesota geneticist Ronald L. Phillips began their quest to physically identify the 21 pairs of chromosomes in domesticated oats, *Avena sativa*.

Eric N. Jellen, then a graduate student at the University of Minnesota, modified a staining technique called C-banding.

C-banding creates light and dark areas on the chromosomes. With Jellen's procedure, a black-and-white video camera attached to a microscope relays images to a computer monitor. The black-and-white images convert to pseudo colors that range from yellow to brilliant red.

"Without C-banding, it would have been impossible to identify any more than seven or eight pairs of chromosomes. But with this technique, we have identified all 21 pairs," says Rines.

This accomplishment helps the researchers identify which oat chromosomes have genes on them that impart disease and insect resistance.

Medical researchers have used the C-banding technique to identify human chromosomes associated with such conditions as Down's syndrome.

In wild oats, Rines and co-workers have been able to identify several new genes that confer crown rust resistance.



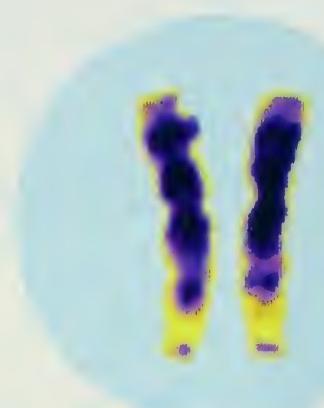
Colorized: On the monitor, geneticist Howard Rines views banding patterns of oat chromosomes. A computer adds color to black-and-white video images captured by a camera mounted on a microscope. (K5391-1) Closeup below shows a Kanota oat chromosome pair with distinct, reproducible C-banding patterns. (K5392-2)

Although these wild species are difficult to cross with cultivated oats, the researchers hope that plant breeders will be able to transfer resistant genes through conventional breeding methods.

Knowing where the genes are "could shave years off breeding new varieties of resistant oats," says Rines. In the past, developing the genetic material—or germplasm—to create new oat varieties with resistance transferred in from wild oats has taken as long as 20 years.

Another key ingredient of this work is being able to look at genetic material that has a chromosome missing. For example, if a plant missing a chromosome also lacks a specific disease-resistance gene, it could well mean that the disease resistance is located on that particular chromosome.

Earlier, Japanese scientists reported identifying 21 oat lines, each appearing to lack one of the different chromo-



somes. Rines and co-workers used their new techniques to take another look at these lines. They found only 12 different ones, instead of the 21 the Japanese had reported.

"Some of the lines the Japanese thought were missing different chromosomes were in fact missing the same ones. But these new techniques should enable us to identify a complete set of 21 chromosome-deficient oat lines needed to locate all the oat genes," says Rines.

The scientists verified their findings by making DNA fingerprints using restriction fragment length polymorphism techniques. This research was partly funded by the Quaker Oats Company.—By Linda Cooke, ARS.

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Detecting Poultry Drug Residues

Chicken's come a long way from the traditional Sunday dinner table. Today it's a star on the fast-food scene, a favorite in frozen dinners, and even competes with hamburger as a ground product at meat counters.

In all, Americans gobbled up about 76 pounds of chicken apiece in 1992, up from 46.7 pounds just a dozen years earlier. To meet this exploding demand, producers hustle new batches of broilers to market every 6 to 8 weeks. And to keep those birds healthy and on schedule, they count on medications called coccidiostats.

"Practically all commercial poultry feed now contains some type of medication," notes biologist Larry H. Stanker of USDA's Agricultural Research Service. "Coccidiostats are primarily effective against coccidiosis, a major disease in chickens."

Broilers begin eating medicated feed as newly hatched chicks, and some of the drugs have Food and Drug Administration clearance for use right up to the day the chickens head for the processing plant. There, chickens are checked to see whether any of the medications are lingering in their bodies—information that in the past could take days to obtain.

That's where Larry Stanker and his colleagues—Ross C. Beier, Marcel H. Elissalde, Jr., and Loyd D. Rowe, Jr.—come into the picture.

"We started working about 2 years ago on developing a quick, accurate means of testing for some coccidiostats in the birds," recalls Stanker, based at ARS' Food Animal Protection Research lab in College Station, Texas.

Of special interest to the researchers was one group of drugs called ionophores, which are not synthetic chemicals, but preparations derived from fermentations of natural microorganisms. Ionophores such as monensin, salinomycin, lasalocid, and narasin account for about \$75 million of the estimated

\$87 million U.S. poultry producers spend annually on coccidiostats.

Also in the research spotlight were a few synthetic medications such as halofuginone and nicarbazine. The Texas researchers actually kicked off their project by pursuing a test for halofuginone.

"The existing test for halofuginone takes at least a couple of days to get the results," Stanker explains. "Our test gives results within a few hours."

To speed up analysis, the Texas team turned to monoclonal antibody probes that work on the same basic principle as a home pregnancy test.

A mouse cell line is manipulated to produce antibodies against the drug in question—as an example, halofugi-

USDA



The fast and accurate detection of residual coccidiostats is important to commercial poultry producers.

none. Then, to check if a chicken's body contains halofuginone, a tissue sample from the chicken is prepared and mixed with the antibodies. A small amount of the drug, bound to an enzyme, is also added.

If the only halofuginone that the antibodies can find to bind is the drug-enzyme combination, then the presence of the enzyme will make the sample turn a vivid color. But if the sample stays clear, this means the antibodies found halofuginone in the bird's tissue.

"So far, we have antibody probes for a variety of coccidiostats, including halofuginone, and a single probe that

will detect both salinomycin and narisin," says Stanker. "But in general, it's one probe for one coccidiostat."

The ultimate practical result of the work, says Stanker, would be a field-portable kit. Toward that end, ARS has applied for a patent on the salinomycin probe. The agency also entered into a cooperative research and development agreement with Neogen Corp. of Lansing, Michigan, that focuses in part on development of commercial versions of test kits for rapid detection of coccidiostats. Neogen plans to obtain a license from ARS for a commercial test for salinomycin as the first in a series of products to be marketed.

"There are a number of reasons for this work," said Stanker. "For example,

feed mills might make a batch of chicken feed, and then a batch of horse feed or turkey feed. However, residual monensin or salinomycin would be extremely toxic to horses, and salinomycin is toxic to turkeys. Feed mills could use a test like this to make sure their feed for species other than chickens is clear of these drugs."—By Sandy Miller Hays, ARS.

Larry H. Stanker is at the USDA-ARS Food Animal Protection Research Laboratory, Rte. 5, Box 810, College Station, TX 77845, phone (409) 260-9306, fax (409) 260-9377. ♦

Jumping Genes Make Genetic Leaps

A pair of eyeglasses, a microscope, a cornfield, and a keen mind were the only tools that plant geneticist Barbara McClintock used in the corn genetics work that earned her a Nobel Prize.

When McClintock began her work, heredity was known to be controlled by genes residing on chromosomes. She surprised the science community by showing that these genes could move—that they were not fixed in linear positions, as had been previously thought.

It was McClintock's jumping genes—technically called transposable elements—that helped ARS geneticist Paul H. Sisco tag a new gene in corn, *Zea mays L.*

"This gene, *glossy-15*, is responsible for producing wax and leaf hairs in corn plants," Sisco says. "And maybe even more important, we've found that it also plays a role in the transition of the plant from the juvenile to adult phase."

Little is known about the juvenile-to-adult vegetative transition phase in plants, so this research could be vitally important—not just for improving corn, but for enhancing the agronomic performance of other crops as well.

Wax-coated corn leaves protect the plant during drought, slowing down moisture loss. Wax can also shield a plant from insects, disease, and ultraviolet irradiation damage from the sun.

In addition to conserving moisture, waxy leaves repel water so that there can be an adequate exchange of gases between the air and the leaf surface. Leaf hairs secrete compounds that can protect corn from pests and disease.

"*Glossy-15* is the first gene associated with juvenility to be cloned in higher plants," Sisco points out. "We're excited because this research shows the

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impact that basic science can have on the practical needs of agriculture."

In some plant species, woody types in particular, the adult phase is the most valuable. The number-one problem in forestry, for example, is getting adult wood, Sisco says.

The new gene won't produce lumber ready for the building trade from an immature oak tree. But it could help speed up the process.

For instance, if *glossy-15* were found in trees species, it could be knocked out. Since this gene prolongs the juvenile

stage, eliminating it would shorten the time it now takes for a tree to mature. And although the *glossy-15* corn gene doesn't make the plant produce corn any faster, it is the first step in learning more about the maturation process.

Sisco says, "Speeding up this transition period may lead to a more efficient way to produce some commodities."

Aided by graduate student Stephen Moose, Sisco cloned the new gene a year ago at the ARS Plant Science Research Laboratory located at North Carolina State University, Raleigh.



► The discoverer: Technician William Brown, who spotted mutant corn plants with the *glossy-15* gene in a research field, looks here for evidence of other jumping gene mutations. (K5321-14)

► Chromosomal breaks: Plant geneticist Barbara McClintock first recognized jumping gene activity in oddly colored corn kernels like these. (K5321-10)

Moose says, "While searching scientific literature on corn genetic research, I kept coming across references to these corn plants that showed wax strips on the third leaf of each plant. Then, when we looked at some mutant corn plants in the greenhouse and realized that several plants showed the same characteristic, I knew I'd found my research mission."

The mutants had been discovered in Sisco's field plantings by William Brown, an agricultural research technician. Seed was collected and grown in the greenhouse for further study.

Intent on finding the gene responsible for the mutations, Moose and Sisco talked with retired ARS geneticist George F. Sprague, the world-renowned scientist who originally discovered the *glossy* gene back in the 1930's [see sidebar]. Sprague was a colleague of McClintock's and shared her passion for corn genetics. There are actually 17 *glossy* genes, but in Sprague's time—and up until the research by Moose and Sisco—it was thought that these genes were responsible just for wax production.

Glossy-15 appears to affect only the leaf surface, or epidermis, of a plant, Moose says. This is significant because plants secrete compounds through their leaf hairs, or trichomes.

The importance of leaf hairs can be seen in recent research on a wild relative of tobacco done by George Buta and ARS colleagues.

Buta, a chemist with the ARS Horticultural Crops Quality Laboratory in Beltsville, Maryland, identified as a group of sugar esters the natural compounds found on the surface of leaves of *Nicotiana gossei*.

An Ancient Crop, Updated

A staple in the diets of the Aztecs, Incas, and other native American peoples, corn as a crop is about 8,000 years old. Today, it is important as a raw material in producing industrial products such as ethanol. A major feed and food crop, both domestic and foreign, corn can only grow as a cultivated crop. It can't survive in the wild. The many superior corn hybrids that we have today may be attributed largely to one man: George F. Sprague.

"In the late 1920's when I started my research, farmers depended on open pollination, saving their own corn seed for next year's crop. This meant that yields were relatively low—I'd say about 30 bushels per acre," muses Sprague. "Nowadays, U.S. average yields exceed 100 bushels."

Before retiring in 1973 as head of ARS Corn and Sorghum Investigations at Beltsville, Maryland, Sprague conducted research on corn genetics that is considered to be among the greatest plant breeding achievements of the 20th century.

He helped develop the scientific principles that provide the foundation for corn breeding and genetics research. Famous for using basic science to increase corn's productivity, Sprague applied his knowledge of corn's genetics to develop varieties that helped the farmer produce more.

"In the early days of corn research, we developed cooperative agreements with Corn Belt states to work on hybridization. Federal researchers were set up in universities in Iowa, Illinois, Indiana, Kansas, Missouri, and Ohio," he says.

The first hybrid corns were introduced in the early 1930's, and by 1940, most of the major corn-producing states were using only hybrids. Sprague says, "By the 1960's, 100 percent of U.S. corn acreage was planted with hybrids."

Stiff Stalk Synthetic, one of the most important corn germplasm sources, was introduced by Sprague. Lines from this source are the ones most widely used in producing commercial corn hybrids today.

In the 1930's, before molecular biology or genetic engineering was recognized as a credible scientific discipline, a young George Sprague discovered a *glossy* gene in corn. From his classical breeding experiments, he knew that this gene was involved in producing wax in the corn plant. But it was not until 1992 that Sprague, then 91 years old, saw the gene cloned and used in genetic testing by another ARS researcher and a student at North Carolina State University.

After 48 years with the Agricultural Research Service and a lifetime of research on corn, George Sprague's work is an excellent example of the integration of basic and applied science.—By Doris Stanley, ARS.

Until recently a Distinguished Professor of Genetics and Plant Breeding, Department of Agronomy, University of Illinois, Urbana, George Sprague now lives in Eugene, Oregon.

These compounds have proven to be environmentally safe for insecticidal use against crop-damaging pests. They are produced by the plant's leaf hairs. The problem is that the plant produces only a very small amount of these compounds.

Could the new corn gene be inserted into the tobacco plant and instructed to produce more plant hairs, and thereby, more of the sugar esters? Maybe, Sisco says.

"Some plants already protect themselves by leaf hair secretions that are toxic to insects," he continues. Certain alkaloids produced in the plant's epidermis are known to deter insect feeding. This could be due to toxicity or to the bitter taste of the compounds.

Glossy-15 opens up new possibilities for genetically engineering pest- and disease-resistance into corn and other plants. Plant resistance is a major hope of growers, since numerous pesticides have been either discontinued or

taken off the market because of potential environmental or health risks.

Impetus for Finding the New Gene

McClintock's discovery of transposable elements was based on her work with the genetics that controlled the purple and bronze color in Indian corn.

She found that when there was a change in corn kernel color, there was a corresponding change in the place where chromosomes tended to break. She hypothesized that this breakage was caused by a "jumping" gene.

A particular gene caused the kernels to be purple. But when the chromosomes broke apart, a different genetic element showed up next to the gene for purple color. So the offspring of that particular kernel produced golden-brown or bronze-colored kernels.

This color change also involved a third gene, which apparently activated the jumping gene. The different-colored kernels were produced by the color gene that did the work; a controlling element, or jumping gene, that told the color gene what to do; and an activator that started the work.

"This is the way we proved that a jumping gene was in *glossy-15*," Sisco says. "When the transposable element is present in the gene, it turns off the gene function, producing sections on the corn plant that have no wax. When the element hops out during leaf development, the gene turns back on, producing waxy sections on the plant." —
By Doris Stanley, ARS.

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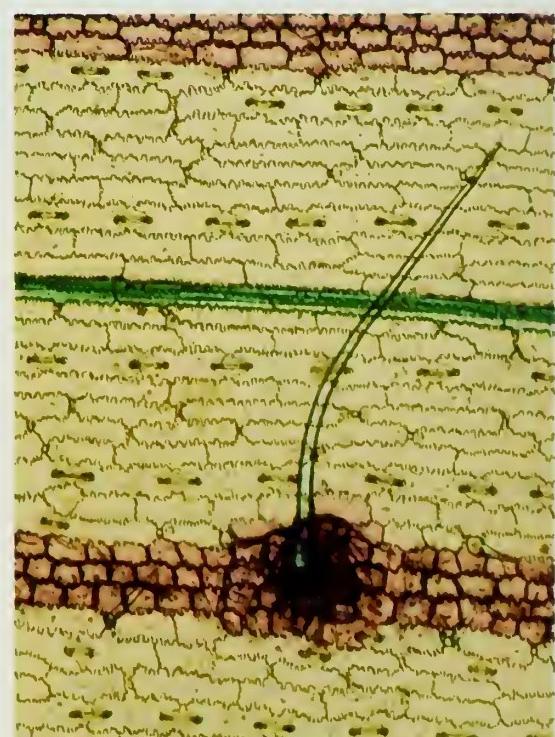
Corn of another stripe: Leaves from a *glossy-15* variegated plant have blue, water-repelling waxy strips where the transposable element has jumped out of the gene and restored normal gene function. (K5323-18)

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Graduate student Stephen Moose prepares to peel and stain a segment of the upper, epidermal layer of a *glossy-15* variegated leaf. (K5322-7)

Close-up: Staining enhances visibility of individual cells and structures in the upper surface of a mature corn leaf. Bluish silica bands make a regular pattern among adult epidermal cells stained aqua. Hairs arising from the corky, purple-stained cell layer protect the plant from pests and diseases.



It's Over for Clover When Gnat Larvae Strike

When agronomist Tim Springer went to work in 1990 at the Agricultural Research Service's South Central Family Farms Research Center at Booneville, Arkansas, it was already October.

So Springer, who planned to study legumes suitable to the Midsouth, thought he'd give next spring's clover crop a head start in the coziness of the greenhouse.

That's when the mystery began.

"The crimson clover began to disappear right out of the pots," Springer recalls. "We took a closer look around the plants and discovered insects—smaller than a fruit fly and shaped like a mosquito."

Springer and collaborator Chris E. Carlton of the University of Arkansas' entomology department identified the culprits as dark-winged fungus gnats, *Bradysia impatiens*. Subsequent greenhouse tests at Booneville have shown that larvae of this tiny pest have an oversized appetite for clover seedling roots.

Adult gnats lay their eggs on the soil surface near the clover seedling. As the eggs hatch, emerging larvae feed on the plant roots. To make matters worse, the wounds they create on the roots may also be wide-open doors to secondary infections from microorganisms in the soil.

"Lack of persistence of forage legumes in pastures is a problem throughout the United States," says Springer. "In some areas, stand reduction of legume species may be attributed in part to fungus gnats, for we have reared fungus gnat species from field-collected sod samples of white clover."

In one greenhouse study, the gnats were turned loose on seven cool-season species of clover. They damaged 96 percent of the kura clover; 92 percent of the white, ball, and arrowleaf clovers; 84 percent of the red

clover; 72 percent of the crimson clover; and 43 percent of the subterranean clover.

"But that doesn't mean planting subterranean clover is necessarily the answer to dealing with these insects," warns Springer. "The subterranean that lived was in such poor condition it wouldn't have survived in the field."

This is not the first time fingers have been pointed at these gnats. In 1969, other scientists hypothesized that the minuscule invaders were responsible for spreading plant diseases among clover. "It was also noted at that time that the gnats could sever an alfalfa seedling root in just 1 hour," he adds.

Producers want to maintain their clover, because as a legume, it can fix, or transform, nitrogen from the atmosphere into a form that plants can use for nourishment.

That's good news—both for grasses in the pasture and for farmers who can reduce their applications of commercial fertilizer.

Clover also offers more protein for grazing animals than do grasses.

"But establishing clovers is really more of an art than a science," Springer notes. "Sometimes you get a good stand, and other times you don't. It can be traced to many factors: how well the seedbed is prepared, weather conditions, or the planting equipment used—and now, possibly to fungus gnats."

They're found on every continent, Springer points out. "They feed on the fungi on decaying plant tissue and in the soil. It may be that these gnats home in on the scent given off by many seeds when they germinate."

"Although this hasn't been confirmed as a significant problem in the field, species of fungus gnats could be the cause of problems with clover stand establishment and reseeding during wet periods."—By Sandy Miller Hays, ARS.

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Red clover.



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Cliff Marshall, of International Absorbents Marketing, Inc., displays a superabsorbent-fertilizer mat system for producing turf grass. In the photo, three stages of seed germination are shown. (K5390-4)

cornstarch to a synthetic one made from petroleum.

The scientific name—saponified starch-graft polyacrylonitrile copolymers—was deemed too cumbersome for informal use so the name “Super Slurper” was coined.

Super Slurper particles can be held in the palm of your hand. They look like flakes of dried, colorless gelatin.

“This discovery took a while to catch on. But once it did, Super Slurper formations made their way into numerous products that touch the lives of agriculturists, horticulturists, environmentalists, and consumers,” says Doane, who is now in charge of plant polymer research at NCAUR.

“Those first-generation slurpers showed promise in human care products ranging from diapers to bandages. But today, agricultural uses for the superabsorbent are surpassing its use in personal care products,” he says.

Super Slurper—Two Decades and Still Growing

Super Slurper, the absorbent material that swells as it gels, is having a birthday. The starch-based product born in 1973 from research at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, is now 20 years old.

Patented in 1976 by ARS chemists William M. Doane, George F. Fanta, Edward B. Bagley, and Mary Ollidene Weaver, now retired, the technology involves grafting a natural polymer in

Companies that were built on the foundation of this technology report that super slurpers can do more than just absorb moisture—their basic job. In agricultural plantings, superabsorbents made with natural starch can potentially reduce nitrate contamination of aquifers, lakes, and streams by nitrogen fertilizer residues.

In early 1992, the Idaho Wheat Commission began seeking information on how to expand nonfood uses of U.S. wheat.

"Our commodity was running behind corn and soybeans when it came to industrial uses," says Mark Samson, administrator of the Idaho Wheat Commission. So Samson and others visited the NCAUR in Peoria to explore possibilities for developing wheat starch's industrial uses.

Doane referred them to a northern Idaho company, International Absorbents Marketing (IAM), owned by the J.C. Marshall family in Smelterville.

An early licensee of the ARS technology, IAM made a superabsorbent from wheat starch. Their product, known as Sta-Wet, is a mixture of the wheat-based superabsorbent and fertilizer that forms a gel. It has a greenish color and looks much like toothpaste. In 1991, they began applying their product along with wheat seed during planting.

The idea of applying fertilizer at the same time as planting seed is a sound one, says company president J.C. Marshall. It can save money and soil compared with running fertilizer equipment in the field after planting.

The absorbent holds both moisture and fertilizer in the soil until needed by growing plants.

"We think that wheat growers in the Northwest could save money on fertilizers, conserve soil, and get good yields. By replacing the cornstarch with wheat, we've gained a new wheat market," says Marshall.

The gelled fertilizer replaces liquid ammonia, which farmers in his area currently apply at an average of 100 pounds per acre, Marshall says. In earlier tests, IAM researchers applied 55 pounds of the gelled fertilizer per acre, or about 40 percent less nitrogen than when liquid ammonia is used.

This aspect of the technology becomes even more important today because of increased environmental concerns about the use of nitrates in agriculture. Farmers are looking for

Superabsorbents made with natural starch can potentially reduce nitrate contamination of aquifers, lakes, and streams by nitrogen fertilizer residues.

new ways to protect soil and water from residual nitrate contamination while still producing bountiful yields.

Marshall says his company plans to modify the drill-planter to place the fertilizer at the side of the seed row. And he says he plans to try Sta-Wet on corn, soybeans, and cotton.

Some other applications for the superabsorbent that IAM is promoting include use in a hydroseeder for planting grasses along highways and

construction sites, in fuel filters to absorb moisture from fuel tanks, and in turf mats for erosion control.

IAM is also supplying a new disease-resistant turf seed to Taiwan, and the company recently shipped its first turf mat to Malaysia.

Another original licensee of the ARS technology, the Super Absorbent Company in Lumberton, North Carolina, is doing well, according to president Ed Kirkland. He recalls that for the first 3 years, he was lucky to gross \$50,000 a year in sales. Today, the company's gross sales exceed a half million annually.

Kirkland's company has three product lines and may soon add another. One provides the absorbent in a seed coating mixture for grass seed; another combines with beneficial microbes in an amendment to enrich soil with nutrients and moisture; and a third works as an absorbent for cleaning up pesticide spills.

James Quinn, president of Industrial Services International in Bradenton, Florida, says they make Terra-sorb water-management polymers. Their product is used to treat bare-root seedlings of nursery trees and shrubs. The polymers keep roots from drying out until the seedlings are replanted.

Vegetable and tobacco roots are also treated with Terra-sorb, and it can be used to grow grass for golf courses. Quinn says that they export about 125,000 pounds of material for use in reforestation. Another 50,000 pounds of the absorbent starch are used to pick up chemical spills.—By Linda Cooke, ARS.

William M. Doane and George F. Fanta are in the USDA-ARS Plant Polymer Research Unit at the National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, IL 61604; phone (309) 685-4011, fax (309) 681-6651. ♦



Plant manager Loren Davis inspects a bag of starch superabsorbent combined with fertilizer. (5389-04)

Breaking Up Is Hard To Do

More like concrete than soil, underground fragipan layers defy penetration.

Two ARS scientists have tackled a big problem that plagues about 33 million acres of U.S. soil and large areas in other countries: how to permeate and break up dense, concretelike subsoil layers that are barriers to plant roots and farm machinery.

Called fragipans, the layers also impede water movement. Once the overlying soil becomes saturated, the water runs off, causing erosion and potentially serious sediment and surface water quality problems.

"Typically 3 to 4 feet thick and about 36 inches below the soil surface in uneroded areas, fragipan layers have been studied by soil scientists since the 1940's," says soil scientist Fred Rhoton of ARS' National Sedimentation Laboratory (NSL) in Oxford, Mississippi. "While we still don't know exactly why they form, we may be close to finding out."

"These layers are so dense, it's like growing plants over bedrock. And their ability to transmit water is so low, they prevent septic systems from working properly," he says.

In 3 years of studying yields of soybeans grown on topsoil of various depths over a fragipan, Rhoton found that erosion can reduce soil productivity to the point that it eventually doesn't pay to grow the crop. With less than a 24-inch topsoil layer, yields dropped by a bushel per acre for every inch of topsoil lost.

"If we can increase rooting depth by just a few inches, crop yields may be substantially increased. This would be a tremendous advantage to farmers."

For the last 2 years, Rhoton has been working with ARS soil scientist Dave Lindbo in the NSL's Upland Erosion Processes Research Unit, studying fragipans in Tennessee, Louisiana, and Mississippi.

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◀ A thin slice: Computer-assisted image analysis helps soil scientist David Lindbo see the composition and microstructural details of a section shaved from a fragipan layer. (K5200-9)

▼ Washout: When water saturates the soil surface, erosive runoff can expose fragipan layers like these being checked by soil scientists Fred Rhoton (left) and David Lindbo. (K5201-8)

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Cutaway view: Soil scientists Fred Rhoton (left) and David Lindbo measure fragipan characteristics in this soil profile. (K5201-18)

Lindbo explains, "These are unique soils covering 12 to 15 million acres in the lower Mississippi River Valley. Because they are among the world's most chemically and structurally complex, we have a lot to learn about them."

"While soil scientists worldwide agree that all fragipans have similar root-restricting and waterflow-inhibiting properties, they don't agree on how they're formed. So we're trying to establish a commonality among fragipans in this region and elsewhere in regard to formation."

Lindbo and Rhoton are working with experts from Ohio State University in Columbus, Louisiana State University in Baton Rouge, and the University of Tennessee in Jackson who have conducted related studies since the 1960's.

"We're using physical and chemical data to identify the role of silica, iron, and clay in fragipan formation," says Lindbo. "We believe they give the soil horizon its unique properties."

"Using x-ray analysis in combination with microscopic analysis of soil structure, we are further describing the nature of the system. The microscopic analysis helps identify patterns of water movement and show how the silica, iron, and clay distributions relate to them."

Adds Rhoton, "When fragipans are disrupted by deep chiseling, soils in the pan tend to reseal and regain their denseness. If high concentrations of iron and silica are responsible for this denseness, we can apply amendments that will displace them or block their negative effects."

The scientists think that adding amendments will speed up the natural breakdown and mobilization of iron and silica that otherwise take thousands of years, improving growing conditions in just a few years without affecting water quality.

"We're not yet sure how deep a section of the fragipan can be affected or how much amendment material should be incorporated to a given depth," says Rhoton. "We'll evaluate amendments such as dairy manure, gypsum from power plants, and pulp mill sludge for their effectiveness at degrading or disrupting the fragipan. The high carbon content and/or high pH of these materials should aid in dissolving the iron and silica." —By Hank Becker, ARS.

Fred Rhoton and David Lindbo are in the USDA-ARS Upland Erosion Processes Research Unit, National Sedimentation Laboratory, P.O. Box 1157, Oxford, MS 38655; phone (601) 232-2938, fax (601) 232-2915. ♦



Pheromone Monitoring Tracks Borers

Keeping mint and sugar beets free of some of their worst insect pests is now a little easier, thanks to ARS scientists.

Entomologist Harry G. Davis and chemist Leslie M. McDonough have identified the sex pheromones—unique scents female moths emit to seduce mates—of two important pests: the mint root borer and the sugarbeet crown borer.

Davis and McDonough are in the ARS Fruit and Vegetable Insect Research Unit in Yakima, Washington.

Synthetic versions of the attractive aromas can lure unsuspecting males to a sticky trap. Moths captured in the trap alert farmers that the enemy is near and it's time to wage a counterattack. This sneaky tactic, known as pheromone monitoring, was first tested in the 1970's and is now used on nearly 100 different crops.

Although the light-brown, nondescript adult moths of the two species don't harm crops, their larval offspring do. Mint root borers chew the underground stems of both peppermint and spearmint. Damage isn't noticeable right away, but come next spring, the field of dark-green, knee-high mint plants will have dry, dead patches. Considered the worst pest of mint, borers have, in some instances, wiped out entire fields.

To better understand the root borer's behavior, Davis spent late nights camped out near a mint field in Washington, observing moths in action. "It seems the peak mating time for these moths falls between midnight and 1 a.m.," says Davis.

Because of that, the scientists had to reset the moths' biological clocks by keeping them in the dark during daylight hours. "That way, we could test the males' response to our synthetic pheromone during the day, instead of in the middle of the night," Davis explains. Technician Pete S. Chapman carried out these indoor tests, using a clear, Plexiglas flight tunnel that allows scientists to observe moths as they fly towards the sex scent.

Before the pheromone could be synthesized, Chapman and chemist Connie L. Smithhisler had to remove the pheromone glands (found on the tip of the female's abdomen) from over 800 moths. McDonough and Smithhisler used a variety of laboratory instruments and tests to determine the mixture's chemical makeup and then recreated it from lab chemicals.

The mint root borer's attractant contains an unusual blend of four chemicals, one of which had never been detected before in Pyralid moths, says McDonough. (Both borers belong to the Pyralid family.)

The team published its findings in 1991, and soon after, a California-based biocontrol company began producing the

lures commercially. McDonough and Davis have obtained a patent on the pheromone.

"The traps may help us determine the best time to apply beneficial nematodes that attack and kill mint root borers," said Joyce Y. Takeyasu, an entomology graduate student at Oregon State University in Corvallis who has tested the traps in peppermint fields throughout Oregon's Willamette Valley.

Together, the traps and nematodes could be an environmentally friendly alternative to currently used chemical insecticides.

A similar scenario is in the works with sugarbeet crown borers. Instead of nematodes, however, researchers plan to

fight borers with *Bacillus thuringiensis*, the commonly used biological pesticide known as *Bt*, says Ed Bechenski, Idaho's Integrated Pest Management coordinator.

Based at the University of Idaho, Moscow, Bechenski tested traps provided by the ARS scientists in Yakima to monitor crown borers in 20 Idaho sugar beet fields. The moth's larvae feed on the beet's crown—the part barely poking up out of the soil. "It's the most devastating when crown borers cut off the crowns early in the season," says

Don Bowers, a field agronomist with the

Synthetic versions of the attractive aromas can lure unsuspecting males to a sticky trap. Finding moths in the trap alerts farmers that the enemy is near and it's time to wage a counterattack.

Amalgamated Sugar Co. in Nyssa, Oregon. The plants die, but it's then too late to replant.

In late summer, the beets—which can reach the size of bowling balls—may still fall prey to the pest. Gnawed beets are vulnerable to bacterial and fungal infections, which means they may rot in storage bins before they're processed into sugar.

"If you'd asked me 5 years ago, I would've said this pest was nonexistent," says Bechenski. Now, we're finding it in every field." Dirty-green in color, crown borers are almost impossible to see unless you get down on your hands and knees in the field. "That's the beauty of these traps—it's so much easier to tell if the pests are present."

Bechenski is currently studying how the numbers of moths found in traps compare to the severity of sugar beet damage. With that data, he'll be able to advise growers when and if to take action against the pests.—By Julie Corliss, formerly with ARS.

For information on patent No. 5,236,715, "Sex Attractant for the Mint Root Borer," contact Leslie M. McDonough or Harry G. Davis, USDA-ARS Fruit and Vegetable Insect Research Unit, 3706 Nob Hill Road, Yakima, WA 98902; phone (509) 575-5970 (McDonough) or (509) 575-5965 (Davis), fax (509) 454-5646. ♦

Powerful Promoter Speeds Genetic Engineering Tests

A fragment snipped from a corn gene is helping biotechnologists worldwide simplify genetic engineering experiments with corn, wheat, rice, barley and sugarcane. Typically, these crops—grains and grasses known as monocots—have been the most difficult to genetically engineer.

The corn gene fragment is a promoter—that is, a portion of a gene that turns it on. Known as the *Ubiquitin-1* promoter, it can be spliced onto lab-designed genes, called reporter or marker genes.

Genetic engineers use reporter and marker genes to get fast feedback about different tactics for shuttling desirable genes into plants. Those genes might make tomorrow's plants resistant to insects or disease, for example. Speedy feedback can help streamline the search for faster ways to get balky monocots to accept new genes, says Peter H. Quail, director of research at the ARS/University of California Plant Gene Expression Center.

Quail and former Center colleague Alan H. Christensen and other co-researchers discovered the *Ubiquitin-1* promoter. Mycogen Corporation's Agrigenetics Division, which funded some of Quail's earlier work with the promoter at the University of Wisconsin, is now seeking a patent.

The *Ubiquitin-1* promoter enhances feedback by boosting the effectiveness of reporter and marker genes. When spliced to a reporter gene, for instance, the promoter strengthens the reporter-derived signal.

"A reporter gene is somewhat like a flare you'd use if you were lost in a wilderness," explains Quail. "It triggers plant cells to manufacture an easily detected signal. The signal could be a faint glow or a blue color that appears when a chemical is added later on in the experiment."

The reporter reveals cells that have either temporarily or permanently taken up whatever new gene scientists are

trying to insert. These cells may be sampled from clumps growing in petri dishes. In successful experiments, some of the clumps will be nurtured into vigorous young seedlings that have the new gene working inside.

"In monocot experiments, you need a strong reporter or marker to find the clusters of cells that have accepted the gene you're trying to give them," says Quail. "The success rate in some monocot genetic engineering tests is still very low, so every cluster that may have taken up the new genetic material is important. With a weak promoter, you may not be able to detect some clumps that are expressing the new genes. Our promoter significantly increases your chances of reliably detecting these transformed cells."

Researchers in Texas worked with the promoter fused to a color-cuing reporter gene. They shot this configuration into sugarcane leaf tissue with a gene gun. The *Ubiquitin-1* promoter, they say, is nearly 100 times stronger than the CaMV35S promoter, the one most commonly used in plant bioengineering experiments.

Scientists in 17 other states and a dozen countries are experimenting with the *Ubiquitin-1* promoter. Those who today hook it to a reporter or marker gene may tomorrow hitch it to a gene that confers a useful trait. Peggy G. Lemaux, who coordinated the Plant Gene Expression Center's genetic engineering of barley experiments—and used the *Ubiquitin-1* promoter in that work—intends to couple it to a disease-resistance gene. Her tests should show if the combination will produce hardy new

barley plants that shrug off yellow dwarf virus, one of the crop's worst disease enemies.—By Marcia Wood, ARS.

Peter H. Quail is at the ARS/University of California Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5900, fax (510) 559-5678. ♦

JACK DYKINGA



Genetic booster: The *Ubiquitin-1* promoter present in greenhouse-grown rice plants examined here by molecular geneticist Peter Quail improves the effectiveness of reporter and marker genes. (K5145-1)

New Seed Separator Gets the Goatgrass Out

Seeds of the dreaded jointed goatgrass weed can be removed quickly from winter wheat seed, using a new invention from ARS.

The device fits on indent-cylinder machines already used by commercial seed cleaners, says Richard A. Caskey of the ARS National Forage Seed and Cereal Research Center located in Corvallis, Oregon.

Wheat seed for planting that is certifiably "weed-free" commands premium prices. But jointed goatgrass seeds, hidden in stemlike segments that give the weed its name, can ruin seedgrowers' profits. If even a single goatgrass segment is discovered in an incoming lot, none of the wheat seed from that lot can be certified, says Caskey. The seed can only be marketed for flour or feed and sold at a loss of 25 to 30 percent.

Caskey invented and patented the seed separator, which he says can be manufactured either as a liner to fit inside today's cylinders or as an entirely new cylinder to fit on existing machines. The invention is intended for cleaning winter wheat harvested from 15 western states now infested with jointed goatgrass.

The weed causes annual losses of about \$150 million to growers, including reduced yields and the cost of shifting from wheat to other, less profitable crops.

A wild relative of wheat, jointed goatgrass so closely resembles the grain crop that for most of the year the two are virtually impossible to tell apart. The seeds, however, are usually distinctive. Jointed goatgrass seeds are longer and more cylindrical than the shorter, rounder seeds of wheat. Inside the rotating cylinder, that difference is crucial.

When tumbled in the cylinder, both seed types briefly lodge in parallel, grooved pockets of the liner's surface.

When the seeds reach a "turnabout" elevation, they begin to topple out of the pockets. The plump wheat seeds escape the pockets first.

However, the cylinder will have rotated to a higher elevation by the time the weed seeds are flung from the pockets. There, they land in a catch-tray that diverts them from the wheat.

"As far as I know," notes Caskey, "there's no other technique as economical for getting jointed goatgrass seeds out of wheat."—By Marcia Wood, ARS.

For technical information on patent No. 5,163,565, "System for Separating Particles on a Rotary Separator," contact David E. Zimmer, Technology Transfer Coordinator, USDA-ARS, Athens, GA 30613; phone (706) 546-3496, fax (706) 546-3401. ♦

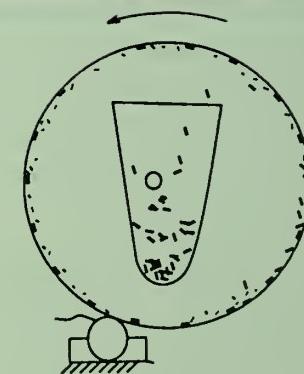
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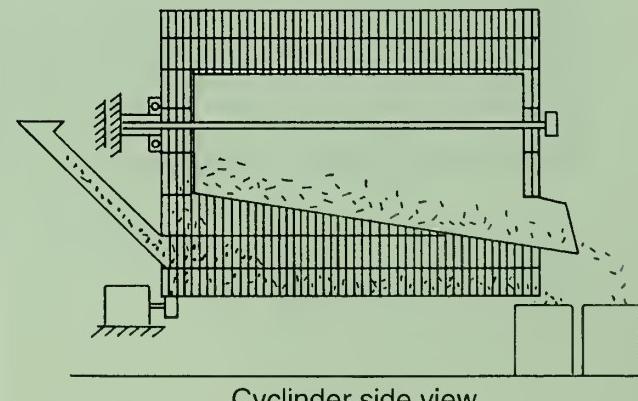
Goatgrass seed. (K5419-11)

SYSTEM FOR SEPARATING SEEDS ON A ROTARY SEPARATOR

Goatgrass seed is separated from wheat as it travels through this rotating cylinder (right). The longer goatgrass seeds stick in small pockets in the cylinder's inner surface and are carried high enough to drop into the center trough. The shorter seeds of wheat stay in the cylinder until the rotation carries them out of the lower other end (bottom).



Cylinder end view



Cylinder side view

Science Update

Scientists Putting Chickens on the DNA Map

Researchers have now placed more than 220 DNA markers on a map of the chicken genome. These gene segments, like highway mile markers, will enable scientists to pinpoint genes for disease resistance, meat quality, or other valuable traits. *Hans H. Cheng, USDA-ARS Avian Disease and Oncology Research Laboratory, East Lansing, Michigan; phone (517) 337-6758.*

Soil Bacteria Guard Wheat From Its Worst Weed Enemy

Soil bacteria may help stop jointed goatgrass, an aggressive weed, on more than 5 million acres of winter wheat. The annual damage tab exceeds \$145 million. But ARS scientists report that three *Pseudomonas* strains colonized goatgrass roots and reduced the weed's growth—without harming wheat. *Ann C. Kennedy, USDA-ARS Land Management and Water Conservation Research Unit, Pullman, Washington; phone (509) 335-1554.*

Electrical Engineering for Genes?

British Technology Group USA, Inc., received a license to use an ARS-patented technique to genetically engineer plants. The technique, electroporation, transfers DNA into pollen via a split-second electrical shock. The shock opens pollen cell pores that DNA can pass through. The modified pollen, placed onto a flower, then makes seed. Scientists say electroporation may be useful for gene-engineering many flowering crops such as alfalfa and corn. *James A. Saunders, USDA-ARS Plant Sciences Institute, Beltsville, Maryland; phone (301) 504-7477.*

New Garbanzos Resist Blight

Two new ARS garbanzo varieties ward off the fungal blight that wiped out half the harvest in the Pacific

Northwest 6 years ago. Dwelley and Sanford garbanzos could help restore Washington and Idaho garbanzo acreage to pre-blight levels. These states, plus Oregon and California, produce most of the nation's garbanzo crop. *Frederick J. Muehlbauer, USDA-ARS Grain Legume Genetics and Physiology Research Unit, and Walter J. Kaiser, USDA-ARS Plant Germplasm Introduction and Testing Unit, Pullman, Washington; phone (509) 335-9521/1502.*

Corn Plants Gang Up To Shade Out Weeds

Growing twice as many corn plants per acre—by planting rows closer together—stifles weeds with shade. The tactic also lets farmers use three-fourths less chemical weed killer without sacrificing yield. ARS scientists planted no-till corn in rows 15 inches apart instead of the usual 30—thus doubling the number of plants per acre. Before corn seedlings emerged, the scientists applied atrazine or metolachlor at one-fourth the usual rate. Currently, corn accounts for 40 percent of the 500 million pounds of herbicide used each year on U.S. cropland. *John R. Teasdale, USDA-ARS Weed Science Laboratory, Beltsville, Maryland; phone (301) 504-5504.*

ARS/Industry R&D Takes Aim With Bio-Based Pest Solutions

Several recent Cooperative Research and Development Agreements (CRADA's) target the commercial potential of genetic engineering or natural biological control agents to solve agricultural pest problems. ARS scientists are working on bio-based CRADA's with—

- biosys, Palo Alto, California, to test several strains of beneficial parasites against sugarbeet root maggot, the crop's major insect pest in the West.

The parasites are tiny, wormlike *Steinernema* and *Heterorhabditis* nematodes. *Garry A. Smith, USDA-ARS Sugarbeet Research Unit, Fargo, North Dakota; phone (701) 239-1350.*

- Pioneer Hi-Bred International, Inc., Johnston, Iowa, to study development of corn that's genetically engineered so the grain will resist beetle and moth damage during storage. If the approach works, corn could be stored longer, with less threat from pests. That would give an export edge to U.S. farmers. ARS is testing the ability of natural proteins to inhibit the pests. Pioneer will seek to transfer into corn the genes that promote the proteins. *Karl J. Kramer, USDA-ARS Biological Research Unit, Manhattan, Kansas; phone (913) 776-2711.*

- EcoScience Corp., Worcester, Massachusetts, to investigate beneficial yeasts that protect harvested fresh apples, pears, and other fruits from molds and rots. This could eliminate spraying chemical fungicides. ARS researchers identified and received a patent for a natural yeast, *Cryptococcus laurentii*, that fends off blue mold, gray mold, and *Mucor* rot. EcoScience researchers are exploring opportunities for mass-producing and marketing the yeast. *Rodney G. Roberts, USDA-ARS Tree Fruit Research Laboratory, Wenatchee, Washington; phone (509) 664-2280.*

- Nippon Zeon Co., Ltd., Tokyo, Japan, to develop a genetically engineered vaccine against poultry coccidiosis. The disease costs U.S. poultry farmers \$450 million annually in medication and lost meat production. Coccidia parasites are becoming resistant to current drugs, and new drugs are costly to develop. *Mark C. Jenkins, USDA-ARS Protozoan Diseases Laboratory, Beltsville, Maryland; phone (301) 504-8054.*

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Upcoming in the **FEBRUARY** Issue

► Barley and other U.S. crops may soon be improved by the efforts of a public/private consortium at the ARS/University of California Plant Gene Expression Center in Albany.

► Gentle, seldom-noticed wild pollen bees are the true workhorses among crop pollinators.

► Range scientists help bring together opposing environmental interest groups to harmonize conflicting points of view.